Long-Term Studies of Fungicide Concentrations in Greenhouses. 1. Technique for Determining Surficial Foliar Residues of Fungicides with Vinclozolin and Triadimefon as Model Compounds

Margareta Papantoni and Lennart Mathiasson*

Department of Analytical Chemistry, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden

Ulla Nilsson

Department of Park and Horticultural Engineering, Swedish University of Agricultural Sciences, P.O. Box 66, S-230 53 Alnarp, Sweden

A new analysis procedure for fungicides has been developed gearing toward estimation of worker exposure in greenhouses. The procedure includes sampling on leaves, glass, and polymers using vinclozolin and triadimefon as model substances and starts with solvation of the fungicides with ethanol from the surface. It proceeds by collection of the solution on disks of cloth, disk extraction with excess of ethanol, evaporation of the solution to dryness, and addition of 1-chlorobutane and ends finally with HPLC determination. Validations of the evaporation step, the desorption of analyte from the disks, and the whole procedure were performed separately. The whole procedure gave recoveries of vinclozolin and triadimefon on leaves of 74% (RSD 4.2%) and 79% (RSD 5.0%), respectively, and on hard surfaces around 90% (RSD *ca.* 6 %) for both substances. The losses in the evaporation and in the desorption step were small, the major losses occurring when the fungicide was collected from the surface. Also, some degradation products of vinclozolin and triadimefon, i.e., 3,5-dichloroaniline, triadimenol, and *p*-chlorophenol, were briefly studied.

Keywords: *Fungicide residue determination; vinclozolin; triadimefon; foliar surface sampling*

INTRODUCTION

After application of fungicides in greenhouses, two types of risks for work exposure occur. One is connected to inhalation of the fungicides and the other to the possible uptake through skin, which may occur in normal work with the plants. Assessment of work environmental risks in greenhouses thus necessitates a knowledge of air concentrations as well as amounts of fungicides on plants accessible for skin contact at different periods of time after treatment.

For air sampling of gaseous compounds or aerosols, relatively simple procedures are available (Garå, 1984). With minor changes these procedures are expected to be suitable for most fungicides. These matters will be discussed elsewhere (U. Nilsson, T. Nybrandt, M. Papantoni, and L. Mathiasson, unpublished results).

Sampling on surfaces is more complicated. One important problem is to determine the amount of fungicide on glass, polymer, or leaf accessible for skin contact. For example, fungicide in deep pores of the leaves should not be sampled when the skin contamination problem is investigated.

Sampling methods for leaves normally involve cutting the leaves into disks of known surface area followed by prolonged extractions usually with water containing a tenside (Gunther et al., 1973, 1974; Iwata et al., 1977). These procedures generally are time-consuming and contain a number of steps, which increases the risk for loss and contamination. Furthermore, large quantities of organic solvents are used. Recently a comparison between different leaf sampling methods was performed including procedures similar to those mentioned above (Bissell et al., 1991). Since the absolute amount of fungicide sprayed on the leaves was not known, this comparison could only be relative.

Most analysis methods developed for vinclozolin and triadimefon have concerned complicated environmental samples, for which there is a need for a highly efficient separation step. Thus, high-resolution gas chromatography has been the method of choice for the final analysis step, generally in combination with tedious workup steps to make the sample suitable for injection. In some cases the sensitivity and the selectivity of the system have been amplified using the electron capture detector (Newsome and Collins, 1989) or the nitrogen sensitive detector (García, 1991). Several multiresidue methods are suitable for the determination of vinclozolin and triadimefon in mixtures, e.g. the one reported by Nickless et al. (1981). Simultaneous determination of triadimefon and its primary degradation products, two triadimenol isomers, is possible using GC and nitrogen sensitive detection (García, 1991).

For situations in which the number of components is limited, HPLC is an attractive alternative, as in the determination of triadimefon in formulation products (Slahck, 1985). The sample preparation is here much simpler compared to the GC methods mentioned above. After spraying in greenhouses with known formulations, the number of components is limited, which makes HPLC an attractive alternative for these types of measurements. Furthermore, an HPLC method is often faster, especially compared with capillary GC. This is of great value when a large number of samples need to be analyzed.

In this paper we describe a procedure for surface sampling and sample workup of fungicides. Special attention is paid to the problem of obtaining reliable

* Author to whom correspondence should be addressed.

0021-8561/95/1443-0157$09.00/0 © 1995 American Chemical Society

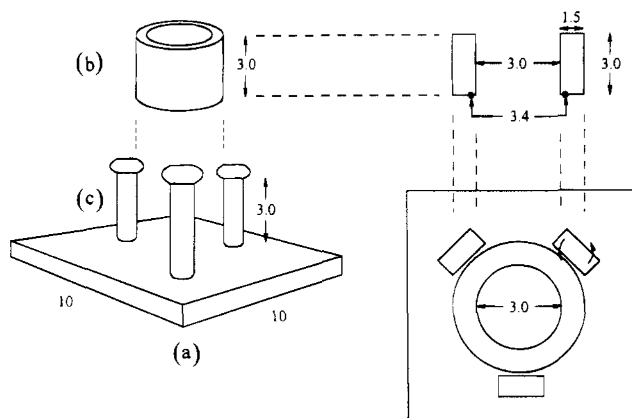

Figure 1. Leaf sampling equipment (dimensions in centimers).

estimates of the risks of skin contamination. The procedure is based on extraction using small amounts of ethanol, which reduces the risk for solvent exposure to laboratory personnel. A normal-phase HPLC system on silica was utilized in the final determination step, since it had previously been shown to give better selectivity in the determination of triadimefon than a reversed-phase system (Slahck, 1985).

The sampling and analysis procedures presented here for vinclozolin and triadimefon on cucumber or tomato plants are presently used in investigations of their concentrations after they have been sprayed in greenhouses and in degradation studies in climate chambers (Nilsson et al., unpublished results).

EXPERIMENTAL PROCEDURES

Equipment. *Sampling and Sample Handling Equipment for the Determination of the Amount of Fungicide on Different Surfaces in Greenhouses.* Figure 1 shows the sampling equipment and Figure 2 the sample handling equipment. The leaf sampling equipment consists of a plate (a) of poly(vinylidene fluoride) (PVDF) polymer. A sample of leaf or of a soft polymer is placed on the plate and the PVDF cylinder (b) is placed on the sample and kept in place by three spring-loaded bars (c). When the knobs of the bars are turned, the cylinder is pressed toward the plate by the springs in the bars. To prevent leakage of ethanol added into the cylinder (see Analysis Procedure and Figure 3), an O-ring is applied in the bottom of the cylinder.

For sampling on hard surfaces (glass or hard polymers) a heavy brass cylinder with the same shape as the PVDF cylinder (b) and with the same inner diameter (but larger outer diameter) is placed directly on the surface. The weight of the cylinder assures leak-tight connection to the hard surface.

The sample handling equipment consists of a steel cylinder (A), a hand-operated piston (B) with an O-ring at the bottom to assure leak-tight conditions, a holder of PVDF with holes (C), and a polyethylene bottle (D). The holder of PVDF gives leak-tight connection between the steel cylinder and the bottle due to the O-rings as shown in the figure. The steel cylinder is firmly attached to the PVDF piece by turning the cylinder so that the joggles (E) fit into the PVDF piece. The steel cylinder has room for up to 40 disk pieces of a fiber cloth consisting of cellulose and cotton (Wettex AB, Norrköping, Sweden). When the piston is pressed, the ethanol solution in the disks is transferred from the cylinder to the bottle. To avoid buildup of an overpressure in the bottle, a small drainage tube (F) is inserted as shown in the figure.

The Wettex disks are punched with a hand punch with proper inner diameter.

High-Performance Liquid Chromatographic System for the Final Analysis. A HPLC pump and a variable-wavelength detector (LKB 2150 and LKB 2151, respectively, LKB,

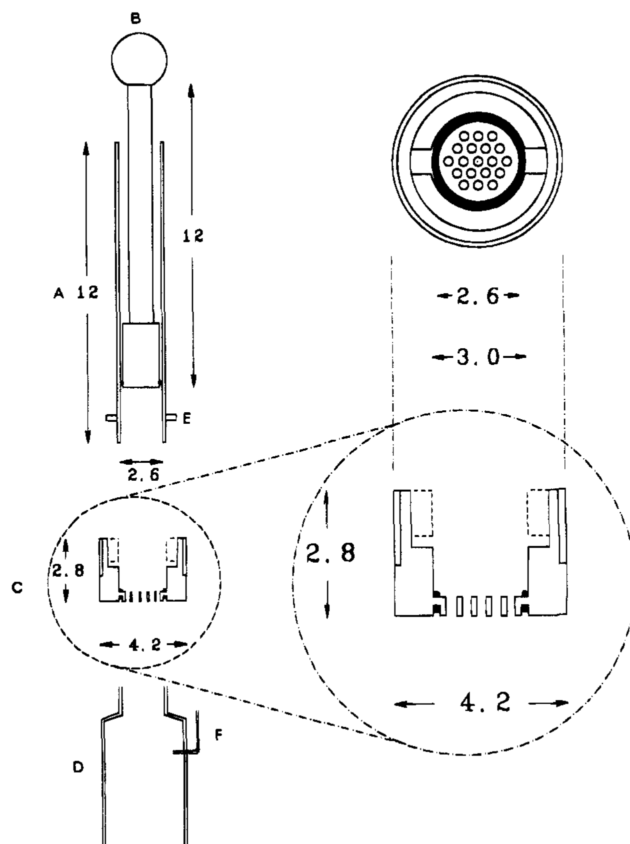

Figure 2. Sample handling equipment.

Bromma, Sweden), an integrator (HP3388, Hewlett-Packard, Avondale, PA), and a HPLC column (Spherisorb S10W 10 μm, 20 cm length, 4.6 mm i.d., Hichrom, Reading, U.K.) were used for the analysis at a flow rate of 0.5 mL/min. Normally the injection volume was 20 μL.

Chemicals. The fungicide formulations were Ronilan FL, an emulsion containing 500 g of vinclozolin/L (E. I. du Pont de Nemours & Co., Wilmington, DE), and Bayleton Special, a powder containing 5% w/w of triadimefon (Bayer, Monheim, Germany). The fungicides and their degradation products were vinclozolin (BASF, Limburgerhof, Germany), 3,5-dichloroaniline (Janssen Chimica, Beerse, Belgium), triadimefon (Bayer), triadimenol (Bayer), and *p*-chlorophenol (Carlo Erba, Milano, Italy). 1-Chlorobutane AR (LabScan, Dublin, Ireland) and ethanol (99%) of HPLC grade were used as eluents.

Analysis Procedure. The analysis procedure is performed as shown in Figure 3. The equipment described in Figures 1 and 2 is used during the sample workup.

At sampling, a leaf was placed on the plate, 0.5 mL of ethanol was added to the leaf surface, and after 20 s, the solution containing the fungicide was swept up with a Wettex disk. Normally each sample consisted of 20 leaves. (Depending on the concentration of the fungicide on the leaf surface, the number of leaves can be increased or decreased.) The disks were then collected in the cylinder, a 5 mL portion of ethanol was added, and the ethanolic solution, containing fungicides extracted from the disks, was pressed into the bottle with the piston. This extraction was carried out four times. The solution collected was evaporated to dryness with a nitrogen flow above the ethanol solution. Before LC analysis, the fungicides were dissolved in 0.5 mL of 1-chlorobutane.

In the validation of the methodology different steps were considered. First, the evaporation procedure needed for solvent change before the final chromatographic analysis was investigated. Second, the desorption step, the transfer of fungicide and possible degradation products from the Wettex disks, was considered. Finally, the whole procedure was tested with a fungicide formulation added to the surface as a water emulsion. Experiments were made for each fungicide and its possible degradation products. Two different solutions were

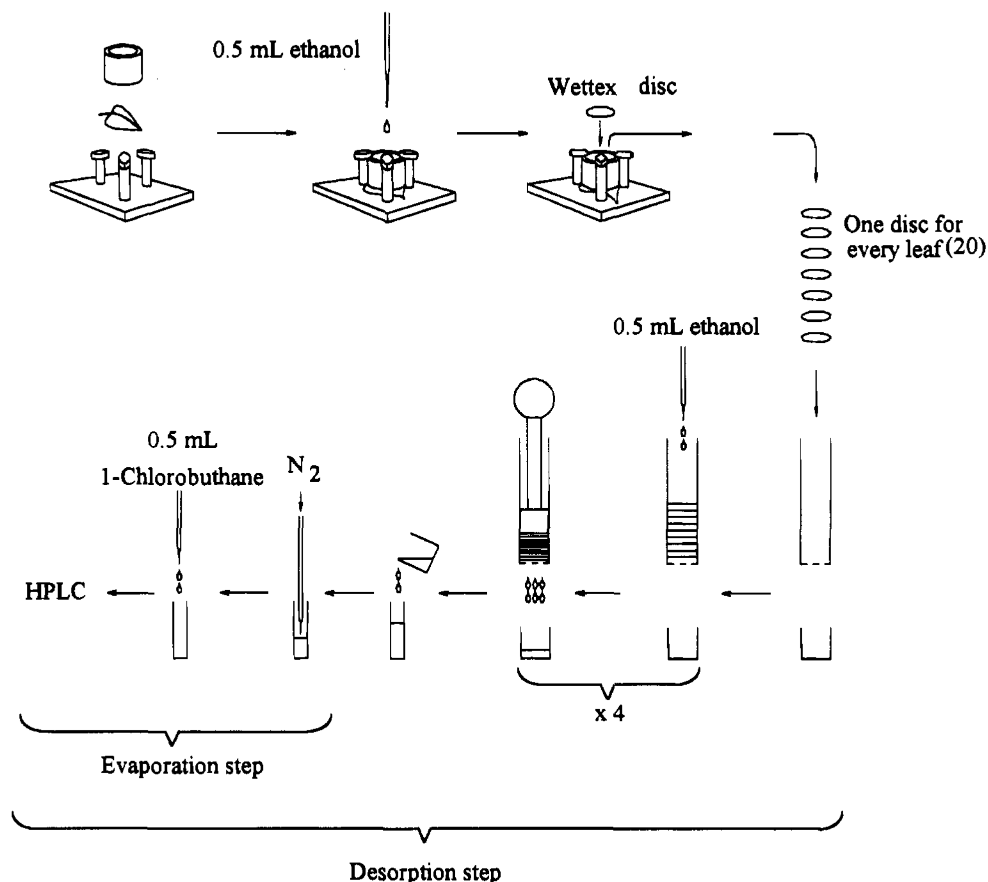

Figure 3. Scheme over the entire analysis procedure.

prepared, one consisting of vinclozolin and 3,5-dichloroaniline and the other containing triadimefon, the two triadimenol diastereomers, and *p*-chlorophenol.

The evaporation step was evaluated with ethanolic solutions containing either vinclozolin and 3,5-dichloroaniline or triadimefon, the sum of triadimenol A and B, and *p*-chlorophenol at concentrations of 5.0, 1.0, 5.0, 10.0, and 1.0 μg/mL, respectively. The desorption step was carried out by dripping 0.1 mL of an ethanolic solution containing the fungicides, respectively, vinclozolin (5 μg/mL), triadimefon (5 μg/mL), and their degradation products 3,5-dichloroaniline (1 μg/mL), the two triadimenol diastereomers (totally 10 μg/mL), and *p*-chlorophenol (1 μg/mL) on 30 Wettex disks. After extraction and evaporation, the sample was analyzed with HPLC.

The total sampling procedure was studied by dripping 0.05 mL of water emulsions, both containing 20 μg/mL of vinclozolin and triadimefon, respectively, on the sampling surface (leaf, hard polymer, soft polymer, or glass). These emulsions were prepared by diluting the formulations Ronilan FL and Bayleton Special with water, and before use the emulsions were ultrasonicated in 15 min. After air-drying, the sample was treated as in the scheme in Figure 3.

When leaf sampling was studied, the emulsion was added to the leaf and the water was allowed to evaporate before the leaf was cut from the plant. This was done to prevent the leaf from drying during the evaporation of the water. Furthermore, to keep the leaves fresh after cutting until further treatment, normally, within the same day, a humidic environment was created by placing the leaves into a plastic box, which was lined with wet cloths and surrounded by a large plastic bag.

RESULTS AND DISCUSSION

Chromatography. *General Remarks.* Figures 4 and 5 show the model compounds and their main degradation products (Del Re et al., 1980; Cabras et al., 1987). With the HPLC system developed, based on silica packing and with a mobile phase of 1-chlorobutane/ethanol in different ratios, it is possible to separate all of the substances shown above, including the two diastereomeric triadimenol compounds.

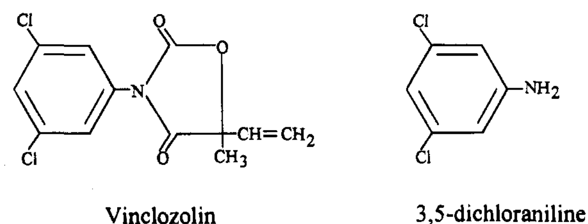

Figure 4. Vinclozolin and its degradation product 3,5-dichloroaniline.

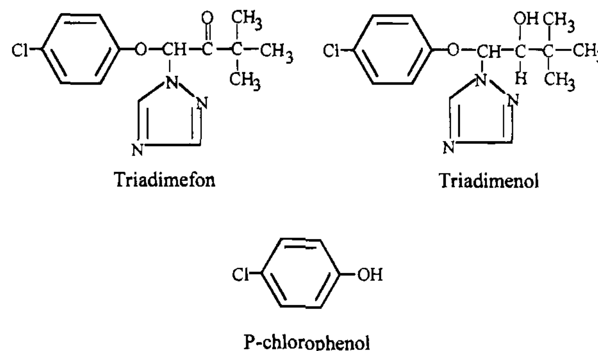

Figure 5. Triadimefon and its degradation products triadimenol, which exists in two diastereomeric configurations (triadimenol A and triadimenol B), and *p*-chlorophenol.

Vinclozolin is preferably determined using UV detection at 240 nm, while higher sensitivity for triadimefon is obtained at 275 nm. For good chromatographic

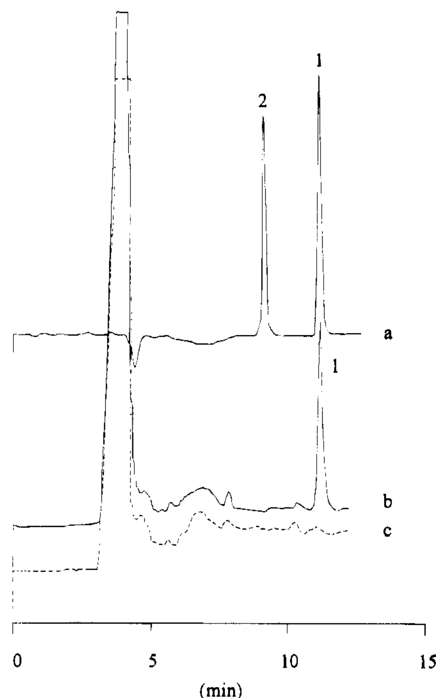

Figure 6. (a) Standard solutions of (1) vinclozolin (10 μg/mL) and (2) 3,5-dichloroaniline (2 μg/mL) in 1-chlorobutane. (b) Sample obtained after applying the formulation Ronilan FL with the active fungicide (1) vinclozolin (8 μg/mL) on cucumber leaves. (c) Blank chromatogram obtained after applying the sample workup procedure to unexposed leaves. Mobile phase, 1-chlorobutane; column, 10 μm silica. i.d. = 4.6 mm, length = 20 cm; flow rate, 0.5 mL/min; UV detection, 240 nm; injection volume, 20 μL.

performance, we have found that it is important to use pure 1-chlorobutane as the solvent for the sample. Already such small injection volumes as 10 μL with ethanol as solvent gave undesirable effects as retention changes and impaired resolution. With pure 1-chlorobutane injection volumes of up to 100 μL could be tolerated without significant changes of retention times and resolution, which is advantageous in trace analysis. An injection volume of 20 μL was chosen for the analysis.

Vinclozolin. With the conditions given under Experimental Procedures the retention times for 3,5-dichloroaniline and vinclozolin, with 0, 1, and 3 vol % of ethanol in 1-chlorobutane as mobile phase, were 9, 6.2, and 5.8 and 11.2, 4.8, and 4.6 min, giving resolutions of 3.7, 2.8, and 2.4, respectively. These values show that any of these compositions could be used. However, a mobile phase of pure 1-chlorobutane is preferred in trace analysis, since the analytes are less affected by disturbing substances, which usually elute close to the front. This is illustrated in Figure 6. Good resolution of vinclozolin from the solvent front is obtained, and the chromatogram at the retention time for 3,5-dichloroaniline is also free from interferents.

Triadimefon. The optimal composition of the mobile phase depends on whether only triadimefon or if degradation products also need to be simultaneously determined. The variation of retention time and resolution with the composition of the mobile phase is shown in Table 1. In the procedure developed by Slahck triadimefon and *p*-chlorophenol were determined on a silica packing with a mobile phase of 1% ethanol in 1-chlorobutane (Slahck, 1985). Table 1 shows that it is possible to simultaneously determine all components considered using 2% ethanol at the cost of a long

Table 1. Retention Times and Resolution between Adjacent Components for a Mixture of Triadimefon (t-fon), Triadimenol A (t-nol A), Triadimenol B (t-nol B), and *p*-Chlorophenol (pcp) Using a Mobile Phase with Different Concentrations of Ethanol (EtOH) in 1-Chlorobutane (CLB)[a]

EtOH/CLB[b] volume %	retention time/min				resolution	
	pcp	t-fon	t-nol A	t-nol B	t-fon/ pcp	t-nol A/ t-nol B
1	7.3	10.4			6.2	
2	7.0	7.4	30.4	42.4	1.0	6.3
3	6.4	6.6	16.7	22.0	0.8	5.1
5	5.6	5.6	10.0	12.0		3.3
10			6.8	8.0		2.7

[a] Column, 10 μm silica with i.d. = 4.6 mm and length = 20 cm; flow rate, 0.5 mL/min; injection volume, 20 μL. [b] Proportion of ethanol in 1-chlorobutane.

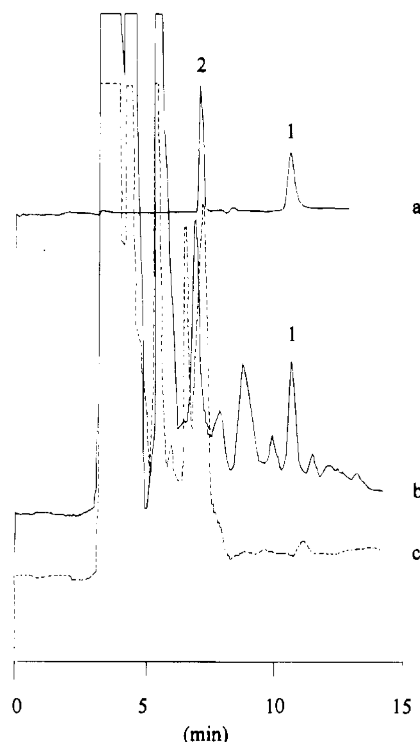

Figure 7. (a) Standard solutions of (1) triadimefon (6 μg/mL) and (2) *p*-chlorophenol (3 μg/mL) in 1-chlorobutane. (b) Sample obtained after applying the formulation Bayleton Special with the active fungicide (1) triadimefon (10 μg/mL) on cucumber leaves. (c) Blank chromatogram obtained after the same sample workup procedure applied to unexposed leaves. Mobile phase, ethanol/1-chlorobutane 1%; column, 10 μm silica i.d. = 4.6 mm, length = 20 cm; flow rate, 0.5 mL/min; UV detection, 275 nm; injection volume; 20 μL.

analysis time. With a large number of samples to analyze, it is generally better to use 1% ethanol for *p*-chlorophenol and triadimefon and 5% ethanol for the triadimenol isomers, additionally giving narrower peaks, resulting in lower detection limits. Although *p*-chlorophenol and triadimefon can be separated with a mobile phase containing *ca.* 2% ethanol, it is, in samples obtained from leaf sampling, in general better to use 1% ethanol. This gives good separation from interfering substances, which normally elute close to the solvent front (see Figure 7).

It is clear that in this case *p*-chlorophenol cannot be determined directly at trace level. The solvent front will obscure the possible *p*-chlorophenol peak. One solution to this problem might be to use an enrichment step with the supported liquid membrane technique (Jönsson and

Mathiasson, 1992). Most of the interferents in the workup procedure of leaves emanate from the leaf matrix, although small amounts of a compound with the same retention time as *p*-chlorophenol come from the Wettex disk. If the disks are extracted once with ethanol and then used in the workup procedure, the background of *p*-chlorophenol corresponds to *ca.* 0.02 mg/m^2 of leaf area compared to expected concentrations of triadimefon after spraying in the order of 1.5 mg/m^2. This is not of major concern, since we have not found detectable quantities in any sample of the long-term stable triadimenol isomers, the first step in the triadimefon degradation. This indicates that the amounts of *p*-chlorophenol one can expect to find will be very small.

Sampling on Surfaces. *Validation of the Evaporation Step.* The sampling procedure results in an ethanol solution, which is not convenient for the final analysis, since injection of only small amounts of ethanol impairs the chromatographic resolution. Accordingly, the ethanol solution must be evaporated to dryness. Such experiments were repeated five times at room temperature with ethanolic solutions containing either vinclozolin and 3,5-dichloroaniline or triadimefon, triadimenols A and B, and *p*-chlorophenol. With nitrogen at a flow rate of 15 mL/s and sample volumes of 15 mL, the recoveries obtained were 98, 75, 103, 100, and 79% for vinclozolin, 3,5-dichloroaniline, triadimefon, the sum of triadimenols A and B, and *p*-chlorophenol, respectively. The corresponding RSD values were 2.0, 12.0, 4.9, 2.2, and 10.2% and the concentrations were 5.0, 1.0, 5.0, 10.0, and 1.0 μg/mL, respectively. Obviously the risk for losses of vinclozolin and triadimefon during the evaporation procedure can be neglected.

Validation of the Desorption Step. In the validation of the desorption step different materials, including polyurethane foam with different pore sizes, were tested for sweeping up the ethanolic solutions containing the fungicides from the sprayed surfaces. With polyurethane foam this step turned out to be difficult. We found a Wettex disk, consisting of cellulose strengthened with cotton fiber, to be satisfactory.

Five samples (analyte concentrations of 5 μg/mL for vinclozolin and triadimefon, 1 μg/mL for 3,5-dichloroaniline and *p*-chlorophenol, and 10 μg/mL for the sum of triadimenols A and B), each consisting of 30 Wettex disks, were processed, resulting in recoveries of 91, 70, 97, 60, and 83% for vinclozolin, 3,5-dichloroaniline, triadimefon, the sum of triadimenols A and B, and *p*-chlorophenol, respectively, with corresponding RSD values of 2.1, 15, 8.4, 5.0, and 6.7%.

Evidently for both vinclozolin and triadimefon there are no serious losses in the desorption step. For triadimefon the recovery after this step is about the same as after the evaporation step discussed above. For vinclozolin it is somewhat lower but still acceptable. For the two triadimenol diastereomers the total recovery after the desorption step was 60%. The losses come from the desorption step since the evaporation step gave a recovery of 100%. For both 3,5-dichloroaniline and *p*-chlorophenol the losses obviously emanate from the evaporation step, since the figures with the desorption step included are about the same as those given for the evaporation. These results indicate that if the degradation products are present in substantial amounts on the sprayed surfaces, they will be detected. If *p*-chlorophenol and 3,5-dichloroaniline need to be determined with higher precision and accuracy than what can be obtained using a correction for losses during the workup procedure, some changes in the workup procedure are needed. One possibility to decrease the losses during the evaporation step might be to add a base to a solution containing *p*-chlorophenol and an acid to a solution containing 3,5-dichloroaniline, leading to salt formation of these two compounds at dryness. However, the 4-chlorophenate and the 3,5-dichloroanilinium salts would not be soluble in 1-chlorobutane. This would, however, considerably complicate the workup procedure since the parent compounds would then be hydrolyzed and the two metabolites need to be extracted into 1-chlorobutane by adding an aqueous solution with suitable pH as well as the organic solvent to the residue.

Validation of the Whole Procedure. The most critical moment in the whole sampling procedure is the transfer of the fungicide from the surface to the solution to be evaporated.

In a preliminary investigation an ethanolic solution of vinclozolin or triadimefon was added onto a hard polymer or onto a glass surface. After air-drying, 0.5 mL of ethanol was added and the fungicide swept up with a Wettex disk. In this experiment the recoveries were above 90% and no significant variation in recoveries could be found, when the extraction time was varied in an interval between 0.5 and 5 min. This also means that the risk of penetration of fungicide into the hard polymer can be neglected since the results on a glass surface were similar.

However, the extraction from a fungicide formulation might be slower. Therefore, experiments were performed in which water emulsions of Ronilan FL (0.05 mL) containing known amounts (30 μg/mL) of vinclozolin were added onto a glass surface. After air-drying, 0.5 mL of ethanol was added and absorbed by the cellulose disk after waiting times between 10 and 50 s. Three experiments, each including 10 Wettex disks, gave overall recoveries of 85, 93, 80, 75, and 77% after contact times of 10, 20, 30, 40, and 50 s, respectively, and corresponding RSD values of 29, 6.0, 10, 8.0, and 3.0%. Obviously the procedure is very efficient at a contact time of 20 s, and the recovery decreases at larger contact times. The reason for this is probably evaporation of ethanol from the surface, which makes the absorption of fungicide by the cellulose disk less efficient. The recovery in the desorption step of vinclozolin is *ca.* 91%, which should be compared to the 93% found in this experiment. Thus, the losses in the overall procedure occur during the desorption step.

At leaf sampling, when ethanol is added onto the surface, the fungicide starts to dissolve and simultaneously a slow penetration of the ethanol solution into the leaf occurs with a risk that small amounts of the fungicide will be lost. Thus, it is advantageous to choose as short a time as possible, sufficient for solvation of the fungicide. To minimize the risk of losses due to penetration, the shortest time giving acceptable precision (20 s) was used in further experiments.

With an extraction time of 20 s, the recoveries of the sampling procedure were investigated on hard polymer, soft polymer, and leaf surfaces. Water emulsions with a known amount of the fungicide (30 μg/mL) were added on the surfaces. After air-drying, the normal sample workup procedure, described under Experimental Procedures, was applied.

Table 2. Results Obtained for the Whole Analysis Procedure for Vinclozolin and for Triadimefon on Different Surfaces with an Extraction Time of 20 s[a]

surface	vinclozolin		triadimefon	
	recovery (%)	RSD (%)	recovery (%)	RSD (%)
hard polymer	90	6.1	88	5.4
soft polymer	5.2	44	2.0	21
leaf	74	4.2	79	5.0
glass	93	6.0	–[b]	–

[a] Water emulsions (0.05 mL) of Ronilan and Bayleton at a fungicide concentration of 30 μg/mL were used in this experiment. $n = 5$ except for glass, where $n = 3$. [b] Not determined.

The values appropriate in discussions of effects of fungicides after spraying in greenhouses depend on their intended use:

If the amounts of fungicides on different surfaces are to be determined, it is natural to use the values in Table 2 to correct for all losses during the sample workup procedure, including the sweep-up procedure.

If the goal is to estimate the risk for skin contamination, the accessible amount on the surface is interesting. The recovery values on leaves in Table 2 are significantly lower than on hard surfaces. We think that this depends on remaining fungicides in small pores which are not efficiently collected with the Wettex disk. The chosen extraction time (20 s) has been shown above to be sufficient to dissolve the fungicide. Most probably this time is short enough that the risk of ethanolic solution containing the fungicide might penetrate the leaf surface and create losses is negligible. Accordingly, the values given in Table 2 corrected for losses during the evaporation and the desorption step should give a good estimate of the accessibility to skin contact.

Starting with vinclozolin, the losses are 2% in the evaporation step and 7% in the desorption step. We think that with the chosen sampling procedure the remaining losses (*ca.* 19%) to a great extent depend on inaccessible fungicide in deep pores. The results on soft polymer show that most of the fungicides are deposited in the polymer during the sample application process. Thus, with this material used for covering in greenhouses, very little fungicide is accessible for skin contact.

For triadimefon there are no losses during the evaporation step and *ca.* 3% in the desorption procedure. Thus, one would expect recoveries around 97% instead of 88% on hard polymers. The difference may depend on the fact that triadimefon needs a longer time to dissolve in ethanol from the formulation or that the sweep-up procedure is less efficient. To investigate this, the sweep-up procedure was repeated with a new portion of ethanol on the same surface. Four samples treated in this way gave recoveries of 87% in the first sweep-up and 4.5% (RSD 24%) in the second sweep-up procedure. We think that most of the amount of triadimefon found after the second sweep-up step should be accessible for skin contact. For triadimefon on leaves, when the same correction as on hard surfaces is applied, the recovery value is *ca.* 85% ($79 \times 1.045 \times 1.03$), which would correspond to *ca.* 15% of remaining inaccessible fungicide in deep pores in the chosen sampling procedure. The small difference in accessibility between vinclozolin and triadimefon may depend on different properties of the formulation. Ronilan FL is available as a water emulsion and Bayleton Special as a powder.

Quantitation. *Chromatographic System.* Calibration curves were made for vinclozolin and 3,5-dichloroaniline on the basis of five evenly spread concentrations in the concentration range 1–75 μg/mL with pure 1-chlorobutane as the mobile phase. The calibration curves obtained were linear, with correlation coefficients larger than 0.999, and in all cases the confidence intervals of the intercepts at the 95% significance level included the origin.

Similar measurements were made for triadimefon and its degradation products, using a mobile phase with 1% ethanol in 1-chlorobutane for the determination of triadimefon and *p*-chlorophenol and a mobile phase of 5% of ethanol in 1-chlorobutane for the determination of the triadimenol isomers. The correlation coefficients were in all cases larger than 0.999, with the lines passing through the origin.

The precision for the substances considered above in the chromatographic determinations is better than 2% for determinations *ca.* 10 times higher than their detection limits. This means that the largest contribution to the overall precision comes from the sampling and the sample workup procedures.

Detection limits for triadimefon as well as for vinclozolin in the system used depend on the strength of the mobile phase: a stronger solvent gives better signal/noise ratio, provided no interfering peaks appear. Figures 6 and 7 show that the model compounds elute well separated from matrix components. For triadimefon the detection limit with a 20 μL injection volume is *ca.* 1 μg/mL and for vinclozolin *ca.* 0.3 μg/mL, calculated as 3 times the noise. These values can be compared with estimated values immediately after formulations containing the two fungicides are sprayed. For triadimefon, following the guidelines for the formulation used (Bayleton Special), values of 50 μg/mL are expected when using 40 Wettex disks for sampling, and for vinclozolin values of 1200 μg/mL using the formulation Ronilan FL and 20 Wettex disks are expected. Thus, the values for triadimefon are *ca.* 50 times above the detection limit, and for vinclozolin the factor is *ca.* 4000.

Detection limits in pure solutions for possible degradation products of triadimefon and vinclozolin are for triadimenol A *ca.* 8 μg/mL and for triadimenol B *ca.* 2 μg/mL using a mobile phase with 5% ethanol in 1-chlorobutane. For *p*-chlorophenol the value is *ca.* 0.3 μg/mL with a mobile phase containing 1% ethanol in 1-chlorobutane, and for 3,5-dichloroaniline the value is *ca.* 0.1 μg/mL with a mobile phase of pure 1-chlorobutane. Sample workup for the triadimenol diastereomers normally does not introduce interfering peaks, which may influence their detection limits. However, *p*-chlorophenol elutes close to the solvent front, which may increase its detection limit, especially in leaf sampling. The use of an electrochemical detector might in this case drastically improve the situation.

Entire Procedure. Formulation emulsions were added to leaves of cucumber with amounts of analyte (vinclozolin and triadimefon) corresponding to concentrations between 10 and 70 μg/mL, and the whole procedure described under Experimental Procedures was applied. Results are shown in Figure 8, where amounts found have been plotted against added amounts. The correlation coefficients for the lines are 0.990 and 0.989 with intercepts of -0.07 ± 0.95 and -0.33 ± 0.61 for vinclozolin and triadimefon, respectively. Detection limits for the whole sampling and workup procedure calculated from these lines are *ca.* 0.5 mg/m^2 for both vinclozolin and triadimefon.

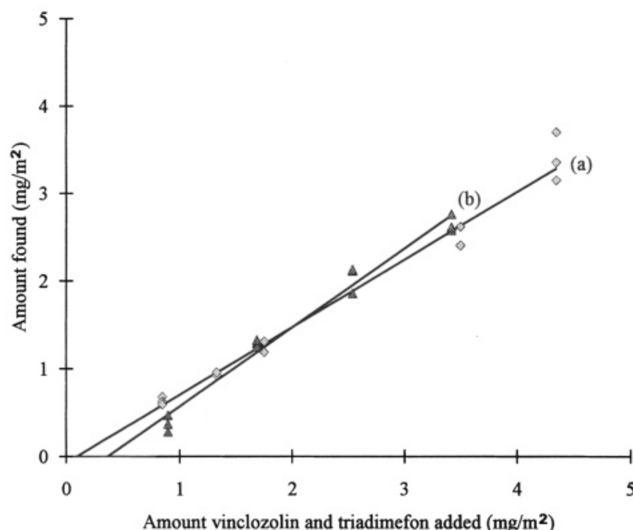

Figure 8. Amounts of vinclozolin (a) and triadimefon (b) found on leaves as a function of the amount added applying the whole sampling and workup procedure.

CONCLUDING DISCUSSION

One objection, which can be raised against a method using organic extractants in leaf surface sampling, is that the solvent may carry external fungicide into the leaf tissue or may extract fungicides which may have penetrated the leaf. The choice of solvent and contact time is important to consider in such an approach. Ethanol was found to penetrate a fresh leaf slowly, in contrast to, e.g., 1-chlorobutane, for which there is a definite risk of loss due to leaf penetration. With the contact time chosen, the risk for diffusive transport of fungicide dissolved in the bulk ethanol solution into the deep pores is judged to be small. Nevertheless, as shown by the results above, not all of the active fungicide in a formulation added onto the leaf is collected, the discrepancy being 10−20%. We think that this is mainly due to the fact that the contact time is too short to allow fungicide in deep pores to be both dissolved and transported to the leaf surface, where it can be soaked up with the Wettex disk. This is, however, in our opinion, an advantage, when the risk for skin contamination is examined.

Another advantage with the advised sampling procedure is the gentle treatment of the leaves. This decreases the risk for interferences, due to extraction of leaf components, in the final analysis step. In the methods based on leaf extraction with water and wetting agents, the leaves may be shaken for hours. However, the sampling and sample workup procedure before the evaporation step is labor-intensive. Up to this point 16 samples can be processed per day within 5−6 h. This leaves enough time to look after the evaporation procedure and to make manual injections in the LC system. Less than 1 day is required to evaporate one sample. With parallel tubes this step is not the limiting factor. The final analysis step takes less than 15 min, and with double injections the sample throughput will be 16 samples per day.

A comparison between the method developed here and methods presented previously (Gunther et al., 1973, 1974; Iwata et al., 1977) is not straightforward. Unfortunately, in these methods no validation of the recovery, for example by using spiked samples, has been made. Normally the number of extractions and the time for each extraction of the leaf sample have been varied until a stable value of the pesticide amount has been reached. This does not ensure that the value obtained corresponds to 100% recovery. Some conclusions can, however, be made by considering the literature data. In the method comparison (Bissell et al., 1991) a moistened cotton gauge was used to sweep up the fungicide. Compared to water−tenside based extractions, values of 60% were obtained, which means that the recovery using moistened cotton gauge cannot be higher than 60%. This can be compared to the method presented here for which recovery values in the order of 75−80% are obtained.

In this paper we have mostly used cucumber plants for the method development. This is not the easiest type of leaf to handle. It has a hairy surface and is much thinner than, e.g., a tomato leaf. This results in a faster drying of the leaf, which increases the risk of solvent penetration into the leaf. Some experiments with triadimefon on tomato leaves, having a more smooth surface and being less sensitive to drying, indicate slightly higher recoveries. We think that this is mainly because it is easier to soak up the ethanol solution containing the fungicide with the Wettex disk. Accordingly, we have reason to believe that the methodology developed here can in general be used for determination of fungicides on leaf surfaces. The procedure developed in this paper might, with minor changes, also be applicable to other pesticides sprayed as water emulsions.

ACKNOWLEDGMENT

We gratefully acknowledge financial support from the Swedish Work Environment Fund. We thank Dr. Jan Åke Jönsson for valuable discussions and Wettex AB Norrköping, for supplying the cloths.

LITERATURE CITED

Bissell, S.; Troiano, J.; Marade, J.; Graham, E.; del Valle, M. Comparison of Sampling Methods for Determination of Pesticide Residues on Leaf Surfaces. *Bull. Environ. Contam. Toxicol.* **1991**, *46*, 397−403.

Cabras, P.; Meloni, M.; Pirisi, F. M. Pesticide Fate From Vine to Wine. *Rev. Environ. Contam. Toxicol.* **1987**, *99*, 83−117.

Del Re, A.; Fontana, P.; Marchini, G. F.; Molinari, G. P.; Rossi, E. Fungicides for Gray-mold Control: A Critical Review of Analytical Methods for Formulation and Residue Analysis. *Residue Rev.* **1980**, *74*, 99−131.

Garå, A. "Pesticide exposure connected with berry cultivation"; Report 1984:18, National Swedish Board of Occupational Safety and Health, Medical Unit in Umeå, 1984.

García-G, J.-E. Improved Method for the Determination of Triadimefon, Triadimenol-A and Triadimenol-B in Wheat Plants (triticum aestivum L.) and Soil Samples by Gas Chromatography. *Fresenius' J. Anal. Chem.* **1991**, *339*, 402−404.

Gunther, F. A.; Westlake, W. E.; Barkley, J. H.; Winterlin, W.; Langbehn, L. Establishing Dislodgeable Pesticide Residues on Leaf Surfaces. *Bull. Environ. Contam. Toxicol.* **1973**, *9*, 243−249.

Gunther, F. A.; Barkley, J. H.; Westlake, W. E. Worker Environment Research. II. Sampling and Processing Techniques for Determining Dislodgeable Pesticide Residues on Leaf Surfaces. *Bull. Environ. Contam. Toxicol.* **1974**, *12*, 641−644.

Iwata, Y.; Knaak, J. B.; Spear, R. C.; Foster, R. J. Worker Reentry into Pesticide-Treated Crops. I. Procedure for the Determination of Dislodgable Pesticide Residues on Foliage. *Bull. Environ. Contam. Toxicol.* **1977**, *18*, 649−655.

Jönsson, J. Å.; Mathiasson, L. Supported Liquid Membrane Techniques for Sample Preparation and Enrichment in Environmental and Biological Analysis. *Trends Anal. Chem.* **1992**, *11*, 106–114.

Newsome, H. W.; Collins, P. Multiresidue Method for Fungicide Residues in Fruit and Vegetables. *J. Chromatogr.* **1989**, *472*, 416–421.

Nickless, G.; Spitzer, T.; Pickard, J. A. Determination of Triadimefon in Grape Juice and Wine Using Capillary Gas Chromatography. *J. Chromatogr.* **1981**, *208*, 409–413.

Slahck, S. C. Liquid Chromatographic Determination of Triadimefon in Technical and Formulated Products. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 586–589.

Received for review September 22, 1994. Accepted October 24, 1994.[⊗]

JF940090P

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1994.